ALGINATE

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Alginates (or alginic acids) are a group of structurally related polysaccharides synthesized by bacteria in the genera *Azotobacter* and *Pseudomonas* (including several plant-pathogenic species) (Fig. 1) and by marine algae (brown seaweeds, *Phaeophyceae*). The polymers have an unbranched irregular structure composed of (1–4)-linked β-D-mannuronic acid and its C5-epimer α-L-guluronic acid (1–3). Bacterial alginates differ from algal alginates in that they are acetylated. The O-acetyl groups are solely on mannuronic acid residues, which sometimes are diacetylated. Alginates produced by *Azotobacter* spp. are more similar to algal alginates in that they contain both polymannuronic acid and polyguluronic acid sequences (referred to as blocks) as well as mixed sequences, whereas the alginates of *Pseudomonas* spp. do not contain polyguluronate sequences. The relative amounts of the different block structures have a profound influence on the physical properties of the polymer, because the presence of homopolymeric sequences of guluronic acid allows for the formation of brittle gels in the presence of calcium.

Alginates produced by pseudomonads are present as extracellular polysaccharides in the form of loosely held slimes. Pseudomonads that have been reported to synthesize alginates are *P. aeruginosa*, *P. chlororaphis*, *P. cichorii*, *P. corrugata*, *P. fluorescens*, *P. mendocina*, *P. putida*, *P. syringae*, and *P. viridiflava*. In contrast, production of alginate as an extracellular polysaccharide by *Azotobacter* spp. is closely associated with the formation of vegetative cysts. *Azotobacter* species reported to produce alginates are *A. vinelandii*, *A. chroococcum*, and *A. beijerinckii* (1). Algal alginates are major structural polysaccharides. Alginates isolated from brown seaweed collected from the oceans have several commercial applications. Alginate salts (called algin) and propylene glycol derivatives of alginate are used in the food, textile, paper, and pharmaceutical industries because of their water holding, gelling, emulsifying, and stabilizing properties (4). There has been intermittent industrial interest in the use of bacterial alginates as substitutes for algal alginates for a variety of commercial applications, but, to date, no bacterial alginates have been commercialized.

The molecular genetics of bacterial alginate synthesis and regulation is the subject of intensive investigation (5). Most studies have centered on alginate production by *Pseudomonas aeruginosa*, because mucoid alginate-producing variants of this bacterium cause chronic, and ultimately fatal, lung infections in patients with cystic fibrosis. In this interaction, alginates are known virulence factors for the bacterial pathogen, mediating adherence to host tissues and subsequent biofilm formation. Bacterial cells present in the biofilms may be protected from phagocytosis and various antimicrobial agents.

The biosynthetic pathway from fructose-6-phosphate to GDP-mannuronate in *P. aeruginosa* has been demonstrated and the structural genes cloned (2). Almost all of the biosynthetic alginate genes are located in one chromosomal region. Fructose-6-phosphate is first isomerized to mannose-6-phosphate by phosphomannose isomerase (*algA*). Mannose-6-phosphate is then converted by phosphomannomutase (*algC*) to mannose-1-phosphate, followed by the synthesis of GDP-mannose via GTP and GDP-mannose pyrophosphorylase (*algA*). The encoding of two enzymes that catalyze nonsequential steps of a biosynthetic pathway by a single gene, as does *algA*, is unusual. Finally, GDP-mannose is oxidized to GDP-mannuronic acid by GDP-mannose dehydrogenase (*algD*). This last step is nonreversible and is thought to be specific for alginate synthesis. Epimerization of mannuronic acid to guluronic acid as well as acetylation occurs on the poly-D-mannuronic acid polymer in the periplasmic space or extracellularly (7). Among the bacterial polysaccharides, the post-polymerization epimerization is unique to bacterial alginates. The structural genes for alginate synthesis and their organization in the plant pathogen *P. syringae* are very similar to *P. aeruginosa* (8). Synthesis of alginate by pseudomonads is increased in response to a variety of environmental stresses, such as nutrient starvation, dehydration, high osmolarity, and during adherence on a solid surface.

There are two chromosomal regions in *P. aeruginosa* that contain genes involved in the regulation of alginate synthesis (2,5,7). The major promoter in the biosynthetic cluster of genes is immediately upstream of *algD*, and

→ 4)-β-D-Man*p*A-(1 → 4)-β-D-Man*p*A-(1 →
2/3
OAc
→ 4)-β-D-Man*p*A-(1 → 4)-α-L-Gul*p*A-(1 →
2/3
OAc
→ 4)-α-L-Gul*p*A-(1 → 4)-α-L-Gul*p*A-(1 →

Figure 1. Structure of bacterial alginates. ManA, mannuronic acid; GulA, guluronic acid (both sugars are in the pyranose form); OAc, O-acetyl group.

it regulates the whole gene cluster as an operon. The regulation of transcription of the *algD* promoter is highly complex and is under the control of the global regulator AlgU, an alternative sigma factor. The gene *algU* (also known as *algT*) is negatively regulated by *mucA* and *mucB*. Several additional, more specific and environmentally responsive regulatory genes, such as *algR*, also affect transcription of *algD*.

The production of alginate by plant-pathogenic pseudomonads when associated with their host plants is thought to be important for virulence. Production of alginate in plants has been demonstrated by its isolation from infected leaf tissue and subsequent chemical confirmation of its identity (8–10). In infected leaves, the production of bacterial alginates may contribute to the prolonged water-soaking of lesions. To prove that alginate is a virulence factor for plant-pathogenic pseudomonads, additional studies are required using highly defined alginate minus mutants.

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